25. (New) The method of claim 20, wherein erythrocyte adenylate kinase activity is determined using an antibody that is specific for adenylate kinase.

26. (New) The method of claim 25, wherein the antibody is specific for erythrocyte adenylate kinase.

REMARKS

Applicants thank Examiners Gabel and Le for the courtesies extended in the telephone interview of August 22, 2001.

Claims 24 - 26 have been added to more particularly point out and distinctly claim the invention. Claim 24 finds support in the specification at least at page 4, lines 10 to page 5, line 5, as well as in the Experimental Details Section at page 9, line 27 to page 11, line 28. Claim 25 finds support in the specification at least at page 5, line 6 to page 7, line 13. Claim 26 finds support in the specification at least at page 5, line 6 to page 6, line 12.

Rejection under 35 U.S.C. 103(a)

Applicants note the arguments made in the Amendment filed on July 26, 2001, and would like to add additional reasons for their assertion that the claimed invention is unobvious over Olsson et al., 1983, J. Appl. Biochem. 5:437-445.

As discussed in the previous amendment, Olsson et al. fails to make the claimed method obvious for the following reasons:

- Olsson never establishes a correlation between hemolysis and erythrocyte adenylate kinase activity;

- Olsson does nothing to provide a correlation between erythrocyte adenylate kinase and erythrocyte hemolysis in a subject, as claimed.

Applicants offer the following additional reasons for the unobviousness of the claimed invention over Olsson.

Although the claims are directed to a method of detecting hemolysis in a subject suspected of having erythrocyte hemolysis, Olsson never evaluated adenylate kinase, let alone erythrocyte adenylate kinase, in a subject that had erythrocyte hemolysis. In the whole blood samples that were tested by Olsson (Table 1, page 443) there was a very low level of adenylate kinase after one day of storage. Based on the knowledge of the current specification, it is clear that the subjects that supplied the blood that was tested by Olsson did not have erythrocyte hemolysis. Thus, Olsson never determined the adenylate kinase activity in a subject that has erythrocyte hemolysis. The skilled artisan would therefore have no indication from Olsson whether an adenylate kinase assay would be useful for the determination of erythrocyte hemolysis in a subject. Additionally, Olsson never states or implies that an adenylate kinase assay could be useful to determine erythrocyte hemolysis in a subject.

Olsson also teaches away from the claimed invention because that publication indicates that platelets can be subject to hemolysis, releasing adenylate kinase. Therefore, the total adenylate kinase measured in whole blood stored for 41 days, as reported in Olsson at Table 1, could have had a significant platelet adenylate cyclase component. Indeed, Olsson found that platelets in storage are particularly susceptible to lysis, releasing high levels of adenylate kinase activity. Thus, the skilled artisan would believe that a subject that was suffering from erythrocyte hemolysis would potentially also have platelet lysis, and that measurement of adenylate kinase activity as performed by Olsson would therefore not necessarily correlate with erythrocyte hemolysis. Indeed, this was confirmed in the instant specification at Table 1 on page 12. As shown therein, total adenylate cyclase activity did not correlate with hemoglobin levels. However, erythrocyte adenylate kinase activity did correlate with serum hemoglobin levels, as

shown in Figure 3. Thus, serum from a patient that had no hemolysis (as determined by a lack of hemoglobin in the serum) could have a significant amount of adenylate kinase activity (see samples 19 and 20). Indeed, in the whole blood samples studied by Olsson (Table 1), the high levels of adenylate kinase detected at 41 days would likely have a significant amount of platelet adenylate kinase. Thus, the Olsson publication provides uncertainty as to whether there is even any significant amount of erythrocyte adenylate kinase in the 41 day old whole blood samples studied therein.

The skilled artisan, upon reading Olsson, would also believe that adenylate kinase levels would be unpredictable in a subject suffering from erythrocyte hemolysis because the subject's metabolism could have a large influence on adenylate kinase activity, for example by removing or inactivating the adenylate kinase from the bloodstream.

Applicants also wish to provide justification for using the term "at least about" in claim 20 to indicate the level of adenylate kinase that would be correlative of erythrocyte hemolysis. As discussed in the specification at page 11, lines 22-28, and shown in Figure 3, the erythrocyte adenylate kinase activity is linear between 1 and 120 U/L, and 1 U/L is proportional to a hemoglobin concentration of 0.005 g/deciliter (DL). This is equivalent to 20 U adenylate kinase activity per liter equal to 1 g hemoglobin per liter. The term "about" is appropriate in this case because the value was determined by estimation based on experimental results, and the term does not specify a limitation of a claim element, but merely an approximate value that indicates hemolysis. The claim is also definite under 35 U.S.C. 112, second paragraph because infringement can be clearly assessed. See MPEP 2173.05(b).

Based on the above discussion, applicants respectfully request reconsideration and withdrawal of the rejection of claim 20 under 25 U.S.C. 103(a) and passage of the claims to allowance. Should there be any additional matters that prevent allowance of the claim, the Examiner is urged to contact the undersigned attorney.

Respectfully submitted,

AMSTER, ROTHSTEIN & EBENSTEIN
Attorneys for Applicant
90 Park Avenue
New York, New York 10016
(212) 697-5995

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By: _____
Elie H. Gendloff
Registration No.: 44,704